MICROBIAL COMMUNITIES OF DEEP MARINE SEDIMENT:

THEIR STRUCTURE OVER TIME AND SPACE

by

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ABSTRACT

Microbial communities of deep marine sediment: Their structure over time and space

Serena A. West

Deep marine sediments make up nearly 70% of the Earth's surface (Morono et al., 2020), and is one of the most understudied environments, with significant funding only beginning circa 2009 (NSF, 2009). This is due in part to the extreme difficulty in accessing the ocean floor, requiring specialized ship-based drilling equipment. These sediments are also a time capsule, preserving the biotic and abiotic materials that were present during sedimentation. As sediment depth increases so does age, potentially covering millions of years of Earth's history. Microbial communities present in these systems must typically survive in low energy, anaerobic, cold, and mobility restricting conditions, leaving very little opportunity for growth. These conditions also preserve environmental DNA, slowly degrading over thousands of years. By comparing intact and degraded DNA as depth increases patterns of microbial depth can be identified, contributing to our understanding of deep marine sediment communities.

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To Holly, who has gone through this journey with me and so she knows she can achieve anything just like her mom. I am forever grateful to my mom Stephanie and sister Chandra, they always knew I could do this and gave me all the love and support. Thank you, John Kirkpatrick, for sharing your data and guidance. And to all the MES staff for their wisdom.

Introduction

Throughout the centuries western science has been anthropocentric centric, focusing on how humans are affected by or how we have affected something. At times little attention has been given to the more remote fields of study that seem to have almost no direct impact on human society. That being said, humans are infinitely curious about the unique and mysterious aspects of planet Earth; extremophiles are one example. The study of deep marine sediments has been one of those neglected fields of research, being extremely remote and requiring specialized equipment to study. The black, cold muck under hundreds of meters of ocean is also one of the most severe environments where life is found.

Microorganisms buried deep in marine sediments have survived, sometimes for millennia or more, with almost no external energy inputs (Bradley et al., 2020; Jørgensen & D'Hondt, 2006). After microbes are deposited on the seafloor as ocean snow or with inorganic detritus, they are slowly buried sealing them in a sediment layer with all the carbon energy they will have for thousands to millions of years (Tanikawa et al., 2018). The energy that is available requires a shift in metabolism, obtaining energy through methane reduction, sulfate reduction, or even the radiolysis of water (Bradley et al., 2020; Jørgensen & D'Hondt, 2006). The lack of available energy combined with low temperatures, typically less than 10°C, forces microorganisms to enter one of three states: metabolically active, dormant, or non-functional (Lomstein et al., 2012). Metabolically active organisms are able to repair damaged DNA and have the possibility of doubling or splitting, although it comes with an energetic cost. Dormant organisms or spores can survive for hundreds of years without expending energy (Lomstein et al., 2012). However, in a dormant state there is no mechanism of DNA repair, ultimately leading to the non-viability of the cell (Johnson et al., 2007). Obviously, the most disadvantageous state is microbial death,

however, the conditions that make life so difficult under the seafloor are also excellent at preserving DNA (Ye et al., 2022). Cold, anoxic conditions may help preserve DNA inside or outside of cells. External DNA (eDNA), outside of a cell membrane, is protected from rapid degradation by the lack of oxygen and by binding to clay or other minerals (Shen et al., 1994). Non metabolically active DNA still undergoes damage and certain types of degradation can give clues to how old the DNA is, the substitution of a cytosine for an uracil for example (Torti et al., 2015). These DNA fragments can be isolated, amplified, and identified in a laboratory, there is potential to identify ancient DNA from species that never lived beneath the seafloor but whose DNA traveled there, whether inside intact cells or not.

Each layer of submarine sediment is a time capsule. Microorganisms cannot effectively migrate to different layers, in a process known as ingrowth (Tanikawa et al., 2018). By identifying and tracking these organisms or their DNA, evolutionary progress can be traced, and major shifts in community structure can be identified. These community shifts could be linked to environmental changes in Earth's past, major disruptive events (i.e., a volcanic eruption or monsoon), or changes in the sediment environment (Lloyd, 2021; Clemens et al., 2015). DNA isolated from deep marine sediment samples can be compared to a reference database and grouped into unique operational taxonomic units (OTUs), which are then associated with a specific microbial grouping. By analyzing OTUs, the patterns of sediment communities emerge, including how many different OTUs were present, their relative abundance, which taxa survived the longest/deepest, etc. Additionally, it can be determined if an OTU is rapidly decaying within a sediment sample, which is done by comparing intact DNA sequences to degraded DNA sequences. This is inferred using paired samples, where both samples come from the same sediment, but one is treated with uracil-N-glycosylase (UNG (+)) which creates cuts in a DNA

strand where an uracil is present (Johnson et al., 2007). Upon heating these cut DNA strands break, preventing amplification and further sequencing. If the paired sample is not treated with uracil-N-glycosylase (UNG (-)), the DNA strains containing uracil are sequenced. The sequencing results can then be compared, treated and untreated, looking for differences in the presence and/or abundance of OTUs. If an OTU has a higher number of UNG (-) readings than UNG (+) that organism is dying off because there is much more degraded DNA within that sediment sample. The focus of this paper is to identify patterns of DNA degradation by uracil substitution within deep marine sediment samples.

Literature Review

History of Deep Subseafloor Research

The ocean floor covers approximately 70% of the Earth's surface with sediment depths reaching over 2.6 km in places (Morono et al., 2020). Representing a massive amount of material and processes that relatively little is known about, it is only within the last 11 years that substantial funding has been invested in furthering our scientific understanding of deep marine sediments (NSF, 2009). As recently as 2020 it was stated that "The physiological status and growth potential of these buried [microbial] communities and, more generally the fractions of these energy-starved subseafloor microbes that are alive, dormant, or dead, have been essentially unknown" (Morono et al., 2020). While commercial ocean drilling has been conducted for several decades, it is only recently that methods to reliably sample subseafloor microorganisms have been developed (Orcutt et al., 2013). One major difficulty in conducting this research is the extreme inaccessibility of deep-sea environments. Vessel-based drilling equipment must first drop through hundreds to thousands of meters of seawater before reaching the seafloor, where drilling commences, and core sediment samples are hundreds to thousands of meters deep (Armbrecht et al., 2019). These samples can span millions of years of sediment accumulation, as seen in Figure 1. Extreme care and planning must be taken to ensure cross-contamination from different sediment layers, seawater, and the surface environment does not occur. A contributing factor to our lack of understanding of the life cycles of deep sediment organisms is the difficulty or impossibility of recreating conditions (i.e., pressure, temperature, and nutrient cycles) in a laboratory setting. Such conditions create extremely slow growth rates for microorganisms and it would take multiple human generations to fully observe microbial behavior *in vivo*.

Since 2003 the International Ocean Discovery Program (IODP), formerly the Integrated Ocean Drilling Program, has been the primary research program gathering sediment samples throughout the world's oceans. It utilizes three research vessels at a time and an international coalition of research institutes (IODP, 2022; Clemens et al., 2016). From 2013 to April 2022, 44 research expeditions have been completed, drilling 559 holes at 197 locations worldwide, with over 70,000 meters of sediment cores recovered (IODP, 2022). The IODP research goals are to better understand climate and ocean change; deep life and biodiversity; deep processes and their effect on Earth's surface environment; and processes and hazards on a human time scale (Bickle et al., 2011). Each research cruise generates massive amounts of data on mineral composition, biogeochemistry, microbiology, paleomagnetism, nutrients, and physical properties throughout each sediment core sample (D'Hondt et al, 2011).

Subseafloor Microbial Communities

When considering microbial growth most people picture the rapid spread of colonies into a surrounding medium. However, in deep marine sediments, the opposite is true. The layers of sediment create a natural archive where very little change or microbial growth occurs. Figure 1 shows the fine layers of silt and clay that have accumulated over 340 m of sediment, the deepest being approximately 75,000,000 years old (75 Ma) (Clemens et al., 2016). During the process of ocean floor sedimentation, cells are buried in fine clay and minerals, immobilizing them. Abyssal clay, for example, is estimated to have a very small pore size of ~0.02 microns (Tanikawa et al., 2018). Microorganisms found in marine sediments have been found to have a size range of 0.25 to 0.7 microns (Kallmeyer et al., 2012), essentially trapping the organisms in the layer they were buried. Increased pressure as sediment depth increases can further compress pore size,

preventing the spread of microbes into adjoining layers, a process known as ingrowth. As depth increases one might expect an initial die-off of terrestrial and aerobic organisms due to cold temperatures (less than 10°C), a lack of oxygen, and low energy availability that severely limit or eliminate microbial mobility and growth (Kirkpatrick et al., 2019). The conditions found deep beneath the seafloor are so limiting that it is estimated that 84% of microorganisms subsist on energy uptake rates lower than previously thought possible for cell survival (1.9×10^{-19} W per cell) (Bradley et al., 2020; LaRowe & Amend, 2015). The sources of energy that are available come from the organic carbon that was present at the time of sedimentation, which becomes exponentially less abundant as depth increases (Jørgensen & Marshall., 2016), as well as products of radioactive decay (Jørgensen & D'Hondt, 2006). Metal reduction, sulfate reduction, and methanogenesis are the major metabolic processes used by microbes to mineralize organic matter for energy production (Jørgensen & Marshall., 2016; Madigan & Martinko, 2006).

Microorganisms have evolved several strategies to survive in such extreme conditions, such as physiological change, dormancy, individual maintenance, and/or cell division times of hundreds to thousands of years (Lomstein et al., 2012). A paper by Kallmeyer et al. (2012) found that most subsurface microbes were very small in size and were nearly spherical in shape, increasing their surface area and

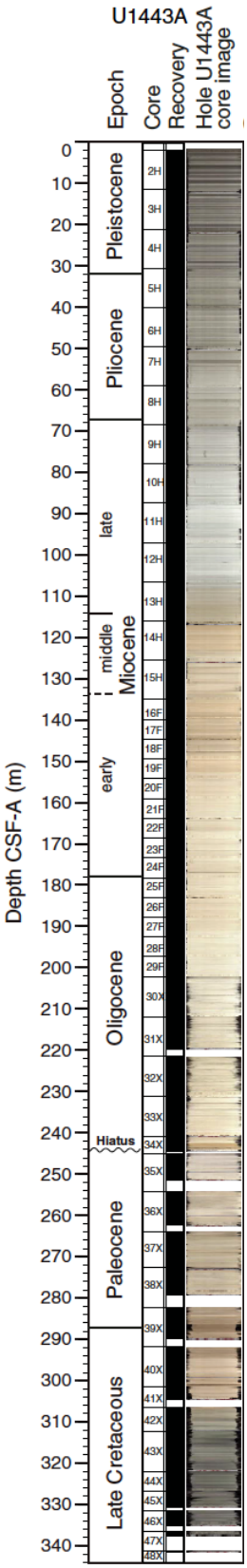

Figure 1. Sediment core from hole U1443A located in the Bay of Bengal (Clemens et al., 2016).

ability to take up nutrients. Other adaptations that increase microbial survival are low membrane permeability, efficient ATP production, and reduced energy requirements (Jørgensen & Marshall, 2016). All of these adaptations lead to an ecosystem that does not require organic matter or solar energy inputs to survive (Jørgensen & D'Hondt, 2006).

An advantage of studying deep marine sediments is evolution can be observed in "real-time." Samples taken at different sediment core depths can represent thousands of years of change, allowing researchers to compare DNA at different time points. An analysis of differences in an organism's DNA sequence can provide insights into evolutionary pressures and conditions (Capo et al., 2022). It is not well understood what evolutionary advantage there is to survive in starvation conditions for millions of years. A common fate of deep marine sediment organisms is to die if sediment temperatures increase to above 80°C near the basaltic basement (Wilhelms et al., 2001). One theory put forward by Jørgensen & Marshall., 2016, suggests that if deep subseafloor microorganisms were suddenly exposed to abundant energy conditions through a major geological event (submarine mudslide, earthquake, etc.), they would have an advantage over other organisms that had not yet entered a starvation state. Generally, it is assumed that organisms stay alive for as long as possible, waiting for the opportunity of passing on genetic material.

As sediment depth increases, the number of microorganisms per cubic centimeter decreases. This shift is drastic, declining to approximately 2.2×10^2 to 5.5×10^6 cells cm^{-3} which is up to seven orders of magnitude less than in energy-rich marine sediments (Morono et al., 2020). Subsea surface microbial communities are derived from the original organisms deposited on the seafloor during sedimentation. Microbial community assemblages change as depth increases with total cell counts and diversity decreasing (Kallmeyer et al., 2012). With the

extremely low rate of cell division, if any, these communities are dominated by organisms with a low mortality rate (Kirkpatrick et al., 2019). Figure 2 shows the reduction in cell abundance and the reduced diversity of operational taxonomic units (OTUs), an OTU is a grouping of genetically related organisms, as depth increases (Kirkpatrick et al., 2019). At each sediment depth, the community assemblage shifts with some OTUs no longer present as conditions change and available energy decreases (Kirkpatrick et al., 2019). Note the large number of unknown OTUs, shaded as grey in Figure 2B, conveying how little is still understood about deep sediment ecosystems (Kirkpatrick et al., 2019).

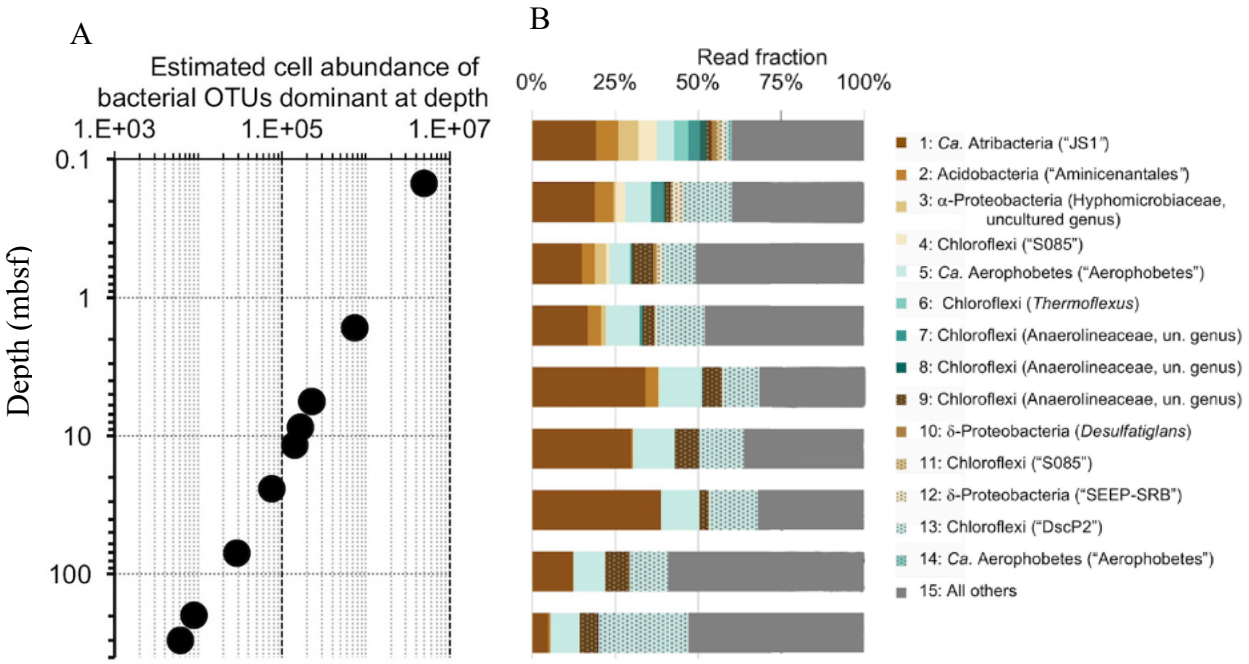

Figure 2. Changes in microbial community assemblages as depth increases, meters beneath the seafloor (mbsf) in the Bering Sea site U1343, depth and cell counts are logarithmic. **A.** The calculated number of bacterial cells as depth increases (cell counts from (Kallmeyer et al., 2012)). **B.** The composition of dominant OTUs near the seafloor to OTUs as sample depth increases (Kirkpatrick et al., 2019).

DNA Pools

Sediment conditions can also preserve environmental DNA, or DNA outside of an intact cell membrane, potentially surviving for thousands of years (Xue & Feng, 2018). By binding tightly to clay or other minerals the exposed DNA strains are protected from rapid deterioration (Shen et al., 1994). The sources of DNA for sequencing contained within a sediment sample generally fall into one of two categories: metabolically active DNA with little damage or degraded DNA. Metabolically active DNA is characterized as being inside an intact cell membrane of a metabolically active cell (Ye et al., 2022). Possible origins of degraded DNA are more varied, it could be ejected from a living cell, within a dormant cell, from a recently dead cell with or without an intact cell membrane, from environmental DNA that was present at the time of sedimentation, or exposed at any time during the millions of years after the original cell was buried.

Advances in metagenomic sequencing and the development of methods to distinguish between active DNA and degraded DNA have increased the understanding of subseafloor microbial communities (Armbrecht et al., 2021). All DNA undergoes damage; loss or substitution of base pairs, breaks in the strand, etc. It is only in metabolically active cells that DNA can be repaired through cellular mechanisms (Madigan & Martinko, 2006). It is the ability to repair DNA that allows microorganisms to live for potentially millions of years. Dormant cells can persist for tens of thousands of years, however, they do not undergo DNA repair leading to eventual non-viability (Johnson et al., 2007). Metabolically active DNA can be isolated from a sediment sample by first performing a wash to remove any DNA not enclosed in a membrane and amplifying only strands of DNA that are over 4,000 base pairs in length (Johnson et al.,

2007). However, it is not possible to determine the age of metabolically active DNA or what genetic mutations have occurred since burial.

When sequencing degraded DNA, the type of damage (i.e., deamination) can be identified and the number of nucleotide substitutions quantified. In general, the more damaged a DNA strand is the longer it is assumed to have been metabolically inactive (Armbrecht et al., 2019). Ancient DNA (aDNA) is of particular interest to researchers, potentially providing a record of organisms that were present in the ancient oceans (Weiß et al., 2020). Typically, aDNA fragments are found in sequences less than 100 base pairs in length or much shorter, depending on environmental conditions (Johnson et al., 2007). Metagenomic sequencing has been instrumental in identifying aDNA, however, new methods of data analysis are still being developed to identify small fragments more quickly and easily (Armbrecht et al., 2021). Sequencing by hybridization, the use of small DNA fragments to complement a damaged strand of sample DNA, is being developed to target organisms of interest in sediment samples. Focusing on a few organisms has led to a 4-to-9-fold increase in the relative abundance when compared to sequencing all present DNA, known as 'shotgun' sequencing (Armbrecht et al., 2021). By targeting an organism of interest its evolution or degradation can be tracked through a core sample, with changes as small as a single base pair substitution identifiable (Capo et al., 2022).

Deamination

One type of DNA damage that occurs despite the preservative nature of deep marine sediment is deamination. As shown in Figure 3, deamination is the loss of an amino group ($-NH_3$) from a nucleotide, the most relevant being the transition of cytosine into uracil (Briggs et al., 2007). Uracil often leads to a substitution in the DNA sequence, from a C:G pair to U:G to U:A to T:A if not repaired (Torti et al, 2015). Both the presence of uracil and an increase in T:A pairs can be identified during DNA sequencing, with more substitutions indicating older DNA. The rate of deamination can vary widely in different environments (Shen et al., 1994), however, the conditions of subseafloor sediments facilitate a slow rate of decay (Briggs et al., 2007).

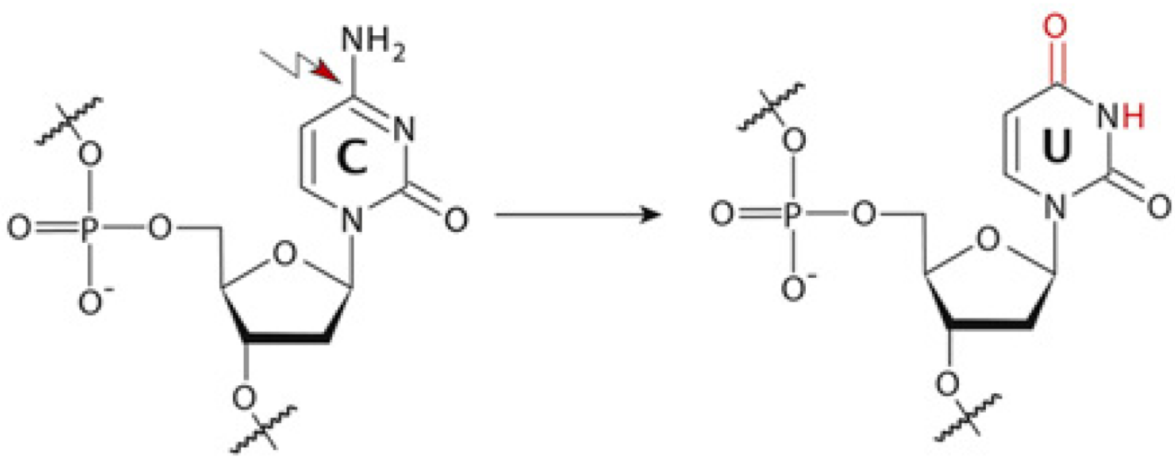

Figure 3. The process of deamination is the removal of an amino group from a nucleotide, in this case, cytosine, converting it to uracil (Torti et al., 2015).

As subseafloor sediment samples have become available scientists have had different research goals in understanding microbial communities. Often the need to distinguish between metabolically active and inactive DNA has been required. As previously discussed, one method to identify intact DNA is to amplify DNA strings of over 4,000 base pairs in length, however, this could include uracil substitutions that do not result in a break (Johnson et al., 2007). An

additional treatment of uracil-N-glycosylase (UNG) to a sediment sample will cause a DNA strand break at an uracil site during the initial heating step of PCR, reducing the overall length and causing it not to be amplified (Johnson et al., 2007). By comparing the sequencing data of a sample untreated with UNG (-) to a paired sample that has been treated with UNG (+) the damaged DNA can be identified (i.e., all sequence data – undamaged sequence data = deaminated sequence data). This is a valuable method of analysis when using data sets that were not initially intended to identify degraded DNA. Such comparisons can result in a pattern of degradation over time, i.e., as depth increases, shifts in dominant microorganisms, and shifts in cellular survival. This is the type of analysis that will be the basis of this thesis.

Methods

Deep marine sediment samples were taken from two locations globally, the Bering Sea and the Indian Ocean, from the research vessel *JOIDES Resolution*. The Advanced Piston Corer (APC) wat the drilling equipment used to extract samples. The Bering Sea samples were taken in 2009 during an Integrated Ocean Drilling Program (IODP) research cruise (Expedition 323 Scientists, 2011). The Indian Ocean drilling study was conducted by the Indian National Gas Hydrate Program (NGHP) in 2007 (NGHP Expedition 01 Scientists, 2007). Both locations were on ocean ridges, near areas of tectonic activity. Table 1. summarizes site information.

Table 1. Sample Site Information

	Bering Sea	Indian Ocean
Site Name	U1343B & U1343E	NGHP-01-14
Date	August 8-10, 2009	July 10-11, 2006
Drilling Method	Advanced Piston Corer	Advanced Piston Corer
Ocean Depth	1962.4 m	906.6 m
Sediment Max Depth	298.9 mbsf	67.12 mbsf
Sedimentation Rate	0.26 m per thousand years	0.15 m per thousand years
Sediment Age at Max Depth	1.15 million years	0.45 million years

At the Bering Sea site, U1343, multiple holes were drilled within approximately 30 m of each other (Expedition 323 Scientists, 2011). Samples for microbiological analysis were taken from holes U1343B & U1343E, see Appendix 1, Table 4. Bering Sea Drill Site Data. Located at latitude: 57°33.4156′N and longitude175°48.9951′W and latitude: 57°33.3814′N and longitude: 175°48.9974′W respectively (Expedition 323 Scientists, 2011). As shown in Figure 4, this site is located on the border of the Bering Sea Shelf and Aleutian Basin and was chosen in part for being an area of high biological production (Expedition 323 Scientists, 2011). The *R/V JOIDES*

Resolution took sediment samples at 7 different locations throughout the Bering Sea from July 5 through September 4, 2009, site U1343 was drilled between August 7 and 12, 2009. Hole B was drilled to 35 meters beneath the seafloor (mbsf) with extra care taken to ensure no cross-contamination occurred, a total of 6 samples were taken. Hole E was the deepest hole at site U1343 with a maximum depth of 780 mbsf, 3 microbiology samples were taken between 81 mbsf to 299 mbsf. Deeper samples could not be taken as the drilling method changed to an extended core barrel (XCB) at 360 mbsf, making cross-contamination highly likely (Expedition 323 Scientists, 2011).

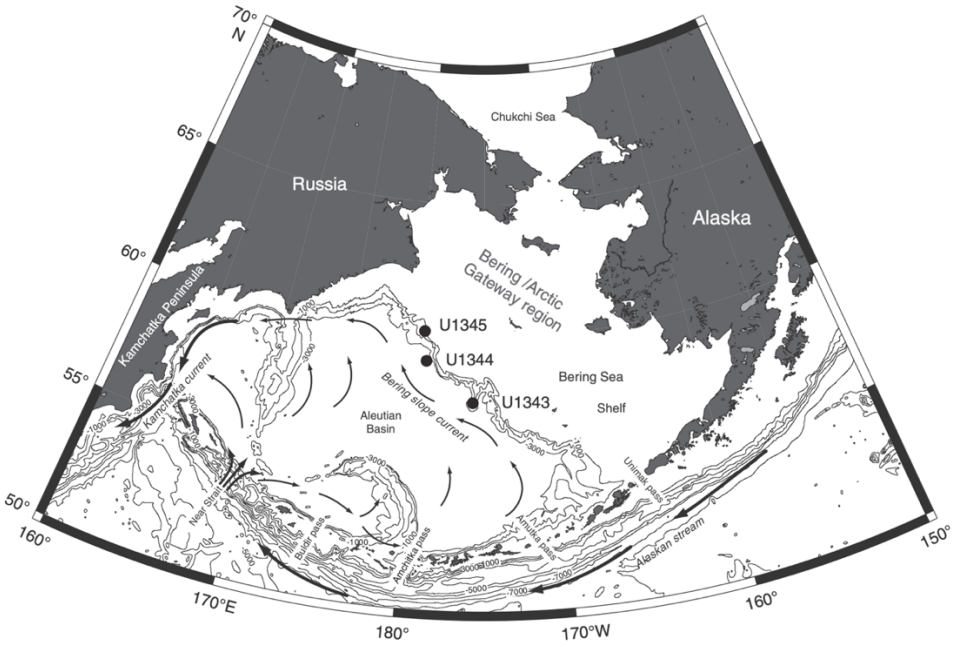

Figure 4. Map of the Bering Sea, IODP Expedition 323 drill sites (Wehrmann et al., 2011)

The Indian Ocean site NGHP-01-14 was one of 15 locations sampled in the Krishna-Godavari Basin during the National Gas Hydrate Program, or NGHP (Kumar et al., 2014). The *JOIDES Resolution* was also the research vessel for this cruise that took place between April 28 and August 19, 2006. Site NGHP-01-14 is located at 16° 03.5577' N, 082° 05.6218' E, and was drilled between July 10 and 11, 2006. Sediment samples were taken to a depth of 175 mbsf with

microbiology samples taken up to a depth of 67 mbsf, 8 samples were taken for DNA analysis (NGHP Expedition 01 Scientists, 2007), Appendix 1, Table 5. Indian Ocean Drill Site Data. Figure 5 shows the 15 sites drilled in the Krishna-Godavari Basin, sites NGHP-01-05 and NGHP-01-15 showed similar sediment accumulation as site 14 (NGHP Expedition 01 Scientists, 2007).

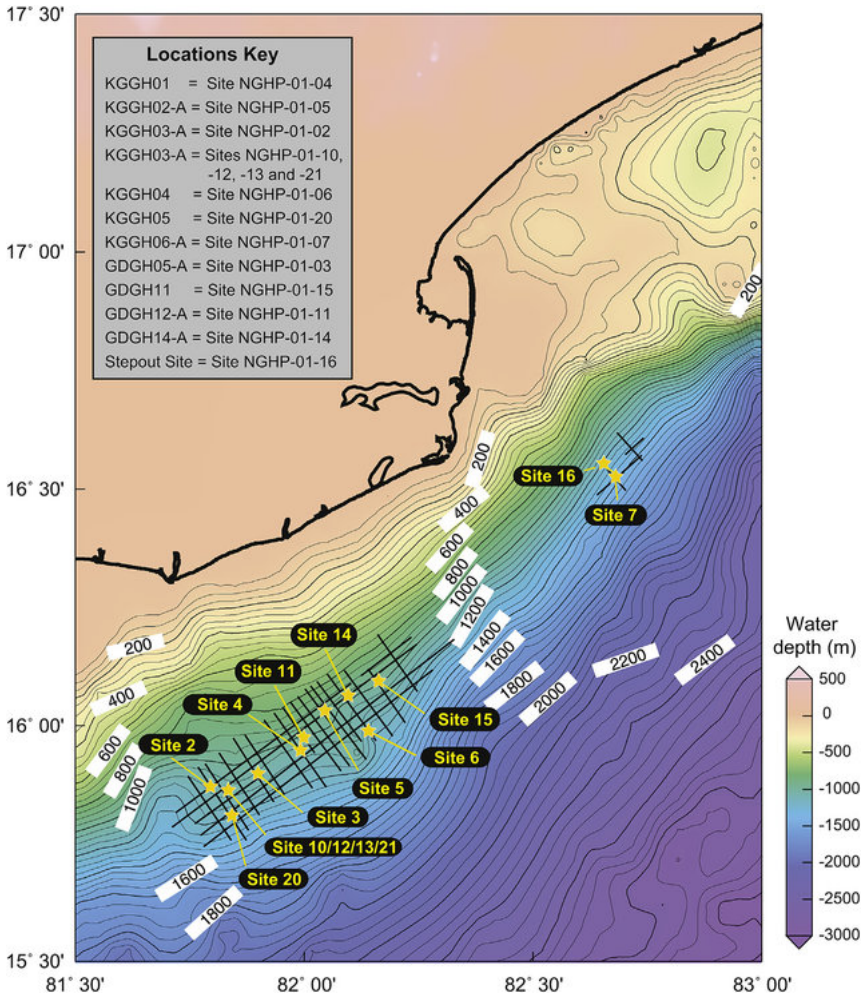

Figure 5. Map of the Indian Ocean, NGHP drill sites in the Krishna-Godavari Basin (Collett et al., 2014).

Sediment samples were transferred and stored aseptically at -80°C until molecular processing could be performed at the University of Rhode Island, RI. Using sterile technique sediments were subsampled for DNA amplification (Kirkpatrick et al., 2019). Samples were

grouped into four clusters: Bering Sea bacteria, Bering Sea archaea, Indian Ocean bacteria, and Indian Ocean archaea. Each of these groupings was also divided into uracil-N-glycosylase (UNG) treated (+) and UNG untreated (-), which caused breaks in the DNA strands at uracil substitution sites (Johnson et al., 2007). Amplification was conducted on hypervariable region v4v5 of the 16S ribosomal gene (rDNA), per convention. The naming convention used for the samples was based on which sediment core they were taken from, the locations they were taken, the Bering Sea or the Indian Ocean, and whether they were treated with UNG. For example, 6H5I+ would be a sample taken from the 6^{th} hydraulic core, in the 5^{th} meter of that core, from the Indian Ocean, and treated with UNG.

DNA extraction and sequencing was conducted as per Kirkpatrick et al. (2019), with the addition of UNG treatments and controls. Briefly, sediment DNA was extracted in triplicate with a bead-beating method including control extracts without sediment. Extracts were cleaned with magnetic beads, washed with ethanol, and re-solubilized with filtered Tris-EDTA before PCR. PCR was conducted in triplicate for every sample, using v4v5 primers for bacteria or archaea as per Huse et al. (2008). PCR products were visualized, cleaned with magnetic beads, pooled, and sent for sequencing at the University of Rhode Island's sequencing facility using Illumina MiSeq 2.0 kits.

Sequencing data was uploaded to the Visualization and Analysis of Microbial Population Structures (VAMPS) database. VAMPS is an open-access website for researchers to upload and compare genomes of microbial DNA sourced primarily from remote and complex microbial communities (VAMPS, 2014). The uploaded data is then compared to the VAMPS sequencing library and grouped into OTUs (operational taxonomic units), the OTUs are then matched to an organism. Depending on the depth of the sequencing library and how common a microorganism

is, the matched organism can be at the domain level down to the strain level, this data set was matched to the genus level or above. Also provided is the number of reads for each OTU in each sample, which are proportional to the pervasiveness of an organism in the sample. After the taxonomy tables were generated, the data was cleaned up and analyzed for patterns of decay associated with sediment depth. Data cleanup consisted of deleting OTUs that were not part of the target domain, i.e., removing archaea, eukarya, and unknown from the bacteria data sets and deleting and removing organisms with zero reads within each pairing. These deletions consisted of less than 1% of the total reads for each data set. Paired samples were then normalized by summing all UNG (+) and UNG (-) reads separately, and then dividing the number of reads for each organism by the total reads. These numbers were then combined to provide the normalized sum, any organism with a normalized sum of less than 0.01 was removed from the analysis, as they did not have a large impact on the results. Normalized UNG (-) reads were then divided by normalized UNG (+) reads for each organism, providing a decay ratio, values greater than 1 indicated more decayed DNA than active DNA within the sample. Table 2. provides examples of this analysis. The values of the decay ratio were then graphed.

$$\text{Single OTU (+) reads} / \text{Total OTU (+) reads} = \text{Norm. UNG (+)} \quad (1)$$

$$\text{Single OTU (-) reads} / \text{Total OTU (-) reads} = \text{Norm. UNG (-)} \quad (2)$$

$$\text{Norm. UNG (+)} + \text{Norm. UNG (-)} = \text{Norm. Sum} \quad (3)$$

$$\text{Norm. UNG (-)} / \text{Norm. UNG (+)} = \text{Decay Ratio} \quad (4)$$

Table 2. Sample Analysis, Bering Sea Bacteria, Sample 1H1B

Organism (OTU)	Reads UNG +	Reads UNG-	Normalized UNG +	Normalized UNG -	Sum Normalized	UNG- / UNG +
C. granulicatella	1	25	9.68×10^{-4}	0.013	0.014	13.284
P. bythopirellula	51	203	0.049	0.104	0.154	2.115
Anaerolineaceae Family	15599	30756	15.093	15.812	30.905	1.048

Total number of reads for 1H1B+ = 103353; total number of reads 1H1B- = 194507

Results

DNA sequencing data generated three taxonomy tables using VAMPS software: Table S1. Bering Sea bacteria, Table S2. Indian Ocean bacteria, and Table S3. Bering Sea archaea, see Appendix 1 for links to the supplemental tables. A total of 16 paired bacterial samples were analyzed, 8 from the Bering Sea and 8 from the Indian Ocean. A ninth sample was taken from the Bering Sea, however, an error occurred during DNA sequencing, and no data was captured. Sequencing was also done on Indian Ocean archaea samples. However, they were not analyzed as the results from the Bering Sea archaea were very limited, and further analysis would not provide additional insights into the degradation patterns of microorganisms as sediment depth increases. This limitation is due to the lack of OTUs generated from archaeal analysis, only 24, very few when compared to the 879 found during the Indian Ocean bacterial analysis. Additionally, the archaeal taxa that were identified in the Bering Sea sample had broad taxonomic groups, rarely going to the genus level. For these reasons, the archaeal analysis will not be considered moving forward. See Appendix 1, Figure 9 for the preliminary S-curve graph of the Bering Sea archaea. Table 3. provides a summary of the taxonomic data generated from both the bacterial and archaeal analyses.

Table 3. DNA Analysis Read Data

	Bering Sea	Indian Ocean
Number of bacterial reads	3,372,229	3,285,604
Number of bacterial taxa	714 to genus level	879 to genus level
Number of paired bacterial samples	8	8
Average number of bacterial taxa per paired sample	79	96
Average number of bacterial reads per pair	6221.4	6069.8
Range of taxa bacterial reads per pair	11- 261070	7-144437
Number of archaeal reads	2,783,322	Data not available
Number of archaeal taxa	24 to genus level*	Data not available
Number of paired archaeal samples	9	Data not available
Average number of archaeal taxa per paired sample	8	Data not available
Average number of archaeal reads per pair	16687.4	Data not available
Range of archaeal reads per pair	10-387701	Data not available

*Most taxa grouped to class level

The taxonomy tables generated by VAMPS created a list of organisms (OTUs) with a corresponding number of reads for each. The number of reads a sample has correlates to the abundance of that organism within the sample, the read data can then be manipulated to show whether an organism is decaying or stable. As shown in Table 2., sequencing data for each paired sample was normalized, and a degradation ratio was generated by dividing the normalized UNG (-) by the normalized UNG (+) for each OTU in that sample. These values were then plotted, and each paired sample generated an S-curve graph with values over 1 indicating more degraded DNA and values under 1 indicating greater amounts of active DNA, see Figure 6.

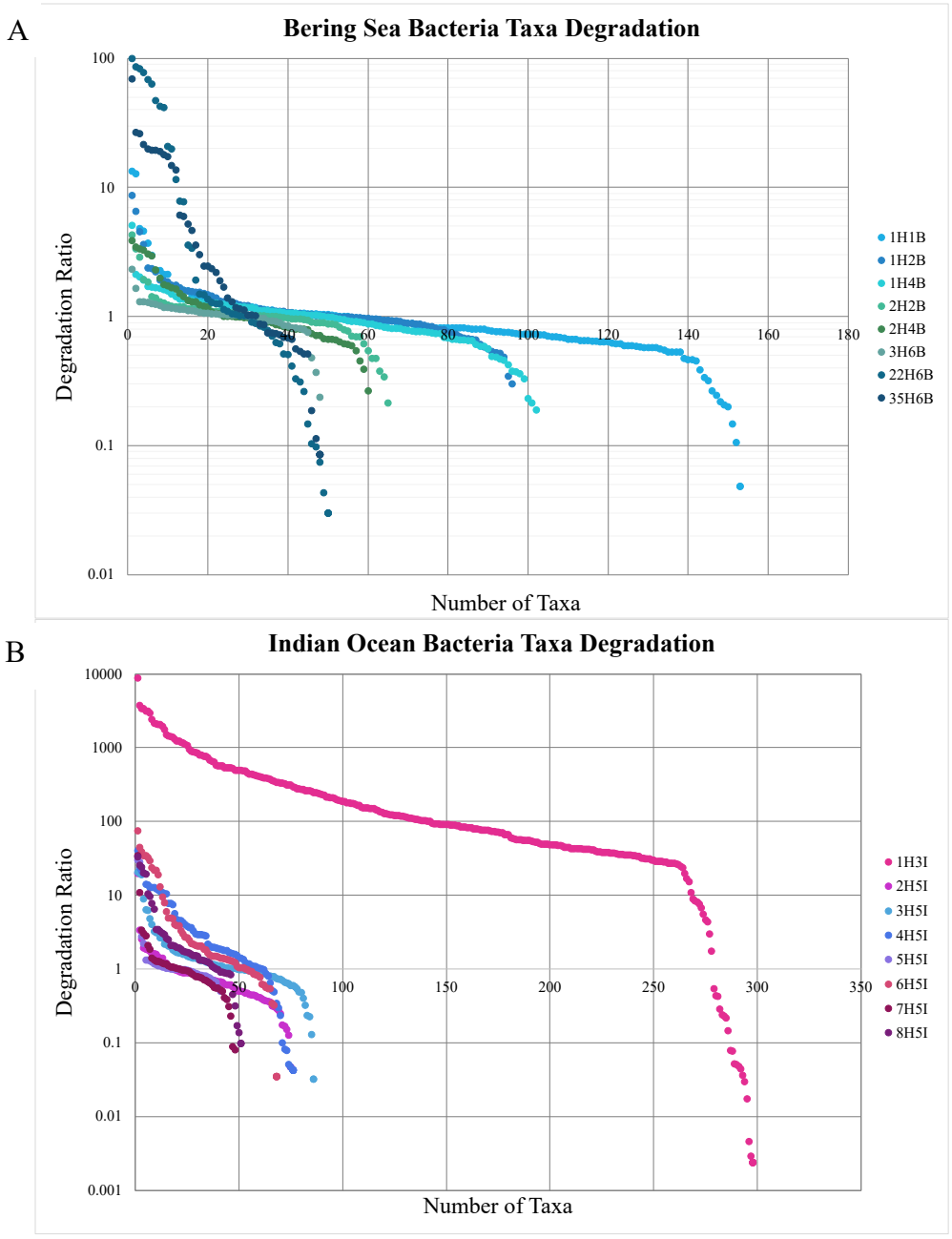

Figure 6. Bacteria S-curves represent the degradation ratio for paired sediment samples. Each dot represents one OTU. A. Bering Sea, B. Indian Ocean

Both S-curve graphs (Figure 6) indicate the highest number of organisms near the seafloor, this is most prevalent in the Indian Ocean with the highest number of organisms from any of the samples, 303 OTUs. As depth increases the number of overall bacteria decreases and

the S-shapes become more elongated, indicating that fewer organisms were able to repair their DNA. Figure 7 shows the degradation of taxa over time, further exemplifying this trend, the decline in OTUs over time, which is an analog of depth. Note that samples untreated with UNG (-) generally have a greater than or equal number of OTUs when compared to the treated sample at any given time point, providing the overall number of taxa lost.

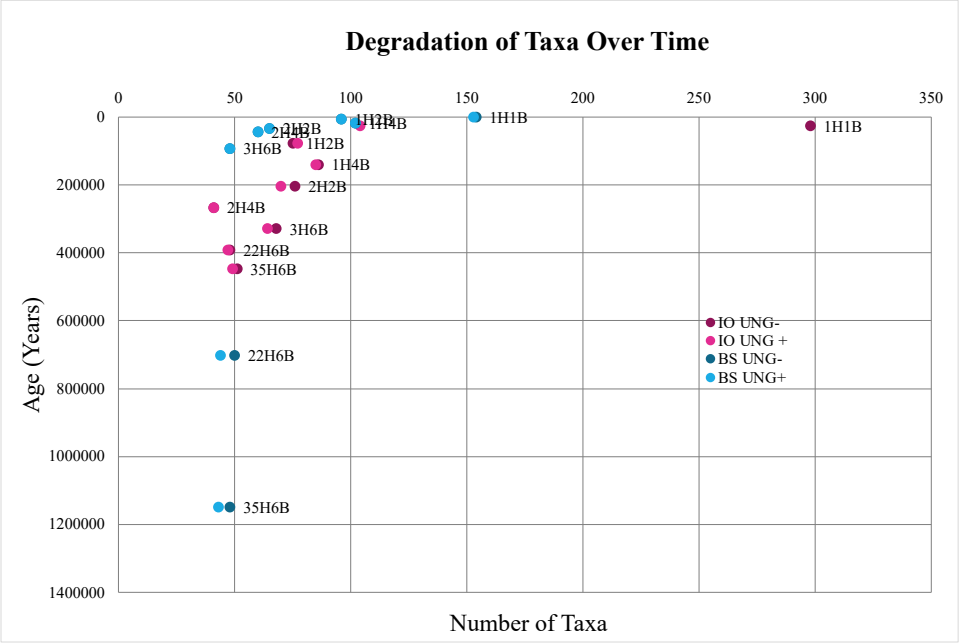

Figure 7. Reduction in the overall number of taxa as age/depth increases. The Indian Ocean samples are represented by paired dots, magenta is UNG (-), degraded DNA, pink is UNG (+), active DNA. The Bering Sea dots are dark blue, UNG (-) DNA, and blue, UNG (+) DNA. Both data sets from the Bering Sea and the Indian Ocean show similar degradation patterns over time (Expedition 323 Scientists, 2011; NGHP Expedition 01 Scientists, 2007). Note, paired samples that overlap appear as one color.

The next step in the analysis was to identify any areas of rapid decay within the sediment column. Figure 8 shows how many organisms were lost at each sample depth. On both graphs, there is a rapid drop in the number of organisms in the first 10 mbsf. The Bering Sea data that goes down much deeper shows a second drop in the number of organisms. In the first 12 mbsf

there was a 62.3% reduction in the number of taxa in the Bering Sea and a 74.6% reduction in the Indian Ocean taxa, 154 to 60 and 303 to 77, respectively.

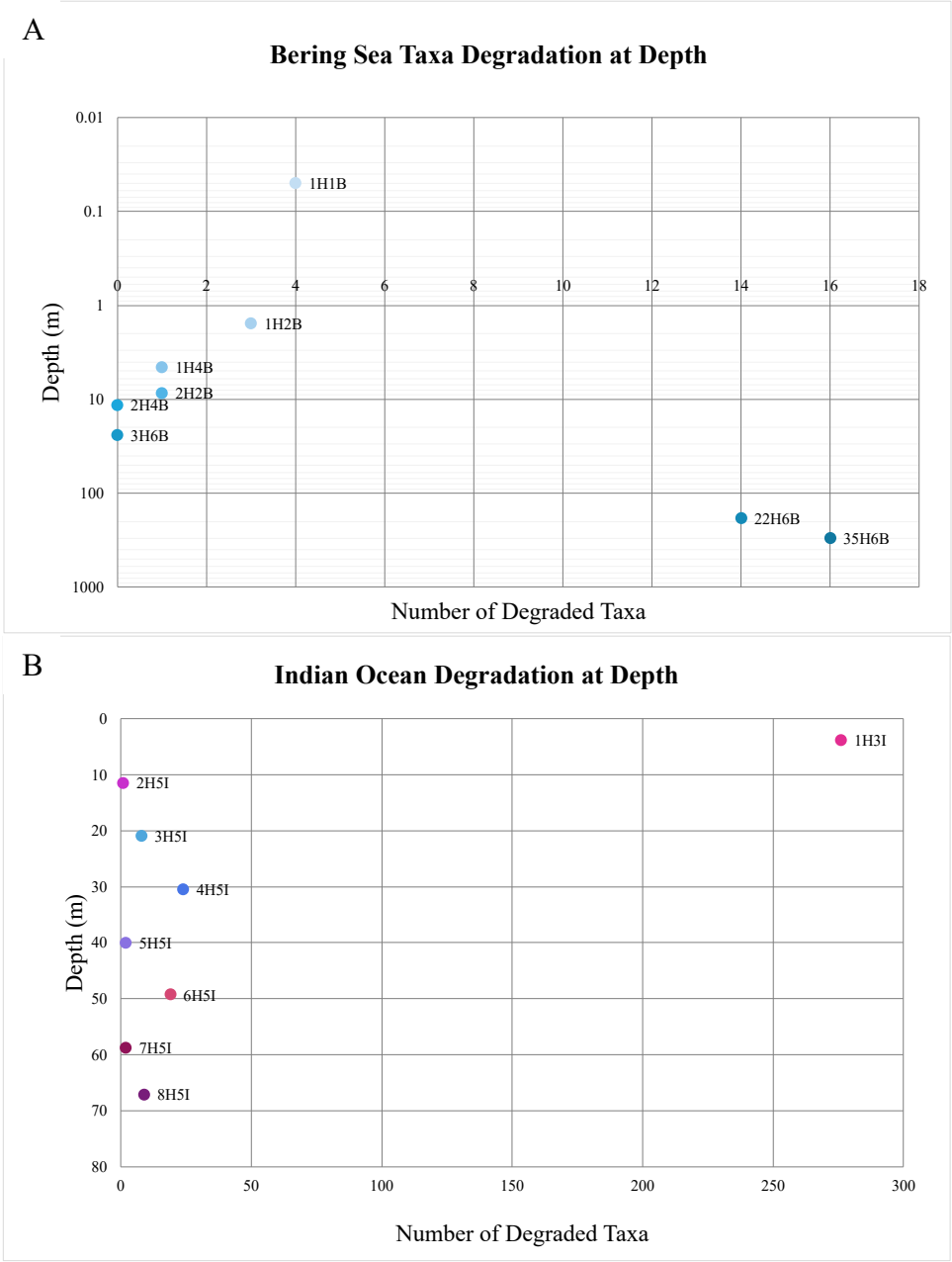

Figure 8. Number of degraded taxa as depth increases. Each point is the number of taxa that were degraded at that depth (i.e., the number of degraded taxa minus the number of non-degraded taxa). Both locations show a reduction at the near surface. A. The Bering Sea shows a second reduction below 100 mbsf while B. The Indian Ocean does not have data deep enough.

Discussion

The general pattern of a large reduction in taxa near the seafloor is consistent with other findings (Kirkpatrick, et al., 2019; Arndt et al., 2013). This is likely due to non-sediment organisms descending through the water column and depositing on the seafloor (Morard et al., 2017). A paper by Walsh et al. (2016) compared the most abundant 100 OTUs in the water column to the 100 most abundant OTUs in the shallow marine sediment (0-10 cmbsf) and submarine sediment (0.25-34 mbsf) and found 41 and 24 overlapping taxa, respectively. Within this data set, there is a much higher number of taxa, nearly double, in the shallowest Indian Ocean sample, 303 taxa, when compared to the Bering Sea, 154 taxa. Many factors affect the number of organisms that settle on the seafloor. In this case, the Indian Ocean site was only 40 km from a major populated landmass, the water depth was 907 m, and warmer surface ocean temperatures, averaged 29.8 °C (Google Maps, n.d., NOAA, n.d.). The Bering Sea location was much further from a sparsely populated landmass, 335 km, was under 1962 m of seawater, and was much colder at 9.6°C on average. (Google Maps, n.d., NOAA, n.d.). All factors that contribute to a much less diverse microbial population in the marine 'snow' deposited in the Bering Sea, as many taxa have already been eliminated before initial deposition. Once these surface organisms reach the seafloor and sedimentation begins, they die off quickly, lacking light, oxygen, or other energy resources (Walsh et al., 2015).

After the initial die-off was observed, the number of taxa that adapted to the subseafloor sediment environment stabilized with very few taxa decaying in sample sites between 10 and 80 mbsf, appx. 32,900 - 447,500 years old. The second major increase in decayed taxa observed in the Bering Sea data, Figure 8A, happened as depth greatly increased, 80 to 182 mbsf, up to 1,149,400 years old. Since no viable samples were taken between these two depths. There were

14 degraded taxa at 182 mbsf and 16 at 299 mbsf, meaning that the number of UNG (-) reads was greater than the number of UNG (+) reads within those taxa. As sediment depth increases the amount of available energy decreases (Bradley et al., 2020; Jørgensen & D'Hondt, 2006), and those microorganisms that were adapted to subseafloor sediment environment eventually succumbed to the extremely low energy conditions, as indicated by the data.

Deep marine sediments are a time capsule, preserving the microorganisms that were present on the seafloor at the time of sedimentation. Identifying organisms that adapt most readily to submarine sediments and are present throughout the sediment core could provide an evolutionary history of that organism. Any changes in the genetic code of a particular taxa would most likely occur at the seafloor or water column, as the rate of cell division within sediments is thousands of years, if doubling occurs at all (Jørgensen & Marshall, 2016). Observing how microorganisms evolve in relation to sediment conditions can provide insights into microbial communities and their role in nutrient cycling. Also, finding points in sediment cores where significant shifts in the microbial community makeup occur could identify times in Earth's past that underwent a major shift in climate.

Conducting further analysis of the archaeal sequencing data could support the findings of this thesis and provide further insights into deep marine sediment microbial communities. However, without more in-depth reference libraries to identify unique taxa other methods must be used. *De novo* clustering is a method that groups individual sequences based on how related they are to each other (Narasingarao et al., 2012). Once the groupings are made the number of taxa can be estimated without assigning an organism. Additionally, the number of reads within each grouping can identify the more abundant taxa. These unidentified taxa groupings can then be analyzed for degradation patterns and consolidated with the bacterial data.

Conclusion

Deep marine sediment microbial communities have a pattern of degradation observed in the two locations analyzed in this paper. The near-seafloor taxa reduction is consistent with the literature (Walsh et al., 2016; D'Hondt et al., 2015; Arndt et al., 2013). However, since many drilling expeditions do not obtain samples suitable for microbial testing greater than 100 mbsf, the second degradation event is not as well documented. Identifying these patterns is just the first step in understanding these communities. Further research into adaptation strategies, nutrient cycling, eukaryotic communities, environmental DNA survival, and microbial evolution are all important areas of research to fully understand the impact deep marine sediments have on global systems.

Understanding the most abundant and arguably alien environment on our planet provides insight into the flow and evolution of Earth over millions of years. Each sediment layer is a snapshot of Earth's past, providing a picture of conditions at that time, going back millions of years (Tanikawa et al., 2018). Microbial community structures are only a small part of the wider picture incorporating, microfossils, geochemistry, mineral composition, nutrients, radiation, paleomagnetism, etc. placing each layer in its historical context contributes to our understanding of the past and how it affects the future.

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Appendix 1

Table 4. Bering Sea Drill Site Data

Site	Core	Org Type	UNG	Depth mbsf	Age Ma	Number of reads
U1343B	1H1	B	+	0.05	0.0002	103353
U1343B	1H1	B	-	0.05	0.0002	194507
U1343B	1H1	A	+	0.05	0.0002	266689
U1343B	1H1	A	-	0.05	0.0002	209389
U1343B	1H2	B	+	1.55	0.0060	264170
U1343B	1H2	B	-	1.55	0.0060	290903
U1343B	1H2	A	+	1.55	0.0060	216915
U1343B	1H2	A	-	1.55	0.0060	174565
U1343B	1H4	B	+	4.55	0.0175	180887
U1343B	1H4	B	-	4.55	0.0175	224900
U1343B	1H4	A	+	4.55	0.0175	200889
U1343B	1H4	A	-	4.55	0.0175	213856
U1343B	2H2	B	+	8.55	0.0329	216050
U1343B	2H2	B	-	8.55	0.0329	181279
U1343B	2H2	A	+	8.55	0.0329	257611
U1343B	2H2	A	-	8.55	0.0329	191111
U1343B	2H4	B	+	11.55	0.0444	160123
U1343B	2H4	B	-	11.55	0.0444	282638
U1343B	2H4	A	+	11.55	0.0444	204142
U1343B	2H4	A	-	11.55	0.0444	163660
U1343B	3H6	B	+	24.05	0.0925	209666
U1343B	3H6	B	-	24.05	0.0925	244204
U1343B	3H6	A	+	24.05	0.0925	126935
U1343B	3H6	A	-	24.05	0.0925	49838
U1343E	9H5	B	+	80.75	0.3106	No Data
U1343E	9H5	B	-	80.75	0.3106	No Data
U1343E	9H5	A	+	80.75	0.3106	121543
U1343E	9H5	A	-	80.75	0.3106	180421
U1343E	22H6	B	+	182.55	0.7021	196771
U1343E	22H6	B	-	182.55	0.7021	217369
U1343E	22H6	A	+	182.55	0.7021	30451
U1343E	22H6	A	-	182.55	0.7021	66225
U1343E	35H6	B	+	298.85	1.1494	137200
U1343E	35H6	B	-	298.85	1.1494	268209
U1343E	35H6	A	+	298.85	1.1494	58115
U1343E	35H6	A	-	298.85	1.1494	50967

Table 5. Indian Ocean Drill Site Data

Site	Core	Org Type	UNG	Depth mbsf	Age Ma	Number of reads
NGHP-01-14	1H3	B	+	3.83	0.0255	268209
NGHP-01-14	1H3	B	-	3.83	0.0255	268214
NGHP-01-14	2H5	B	+	11.45	0.0763	112173
NGHP-01-14	2H5	B	-	11.45	0.0763	159564
NGHP-01-14	3H5	B	+	20.95	0.1397	184706
NGHP-01-14	3H5	B	-	20.95	0.1397	196200
NGHP-01-14	4H5	B	+	30.45	0.2030	99979
NGHP-01-14	4H5	B	-	30.45	0.2030	210991
NGHP-01-14	5H5	B	+	39.95	0.2663	233765
NGHP-01-14	5H5	B	-	39.95	0.2663	273603
NGHP-01-14	6H5	B	+	49.23	0.3282	183264
NGHP-01-14	6H5	B	-	49.23	0.3282	201360
NGHP-01-14	7H5	B	+	58.73	0.3915	252430
NGHP-01-14	7H5	B	-	58.73	0.3915	242328
NGHP-01-14	8H5	B	+	67.12	0.4475	186399
NGHP-01-14	8H5	B	-	67.12	0.4475	212419

Table 6. Supplemental Taxonomy Links

Table Name	Link
S1. Bering Sea Bacteria Taxonomy	https://docs.google.com/spreadsheets/d/1h87s3kPjFf_bn_zRYjP95oVMi5luFEvC3T_SSkCtcaM/edit?usp=sharing
S2. Indian Ocean Bacteria Taxonomy	https://docs.google.com/spreadsheets/d/1tNnkhatz33ulGtaavLRBb5x8XWqOVm55d9Hvrl2OvTg/edit?usp=sharing
S3. Bering Sea Achaea Taxonomy	https://docs.google.com/spreadsheets/d/1wPOIL-mO8QR0eme7JlmtNTfGOGHso9-KZ19ZAOUg0Mk/edit?usp=sharing

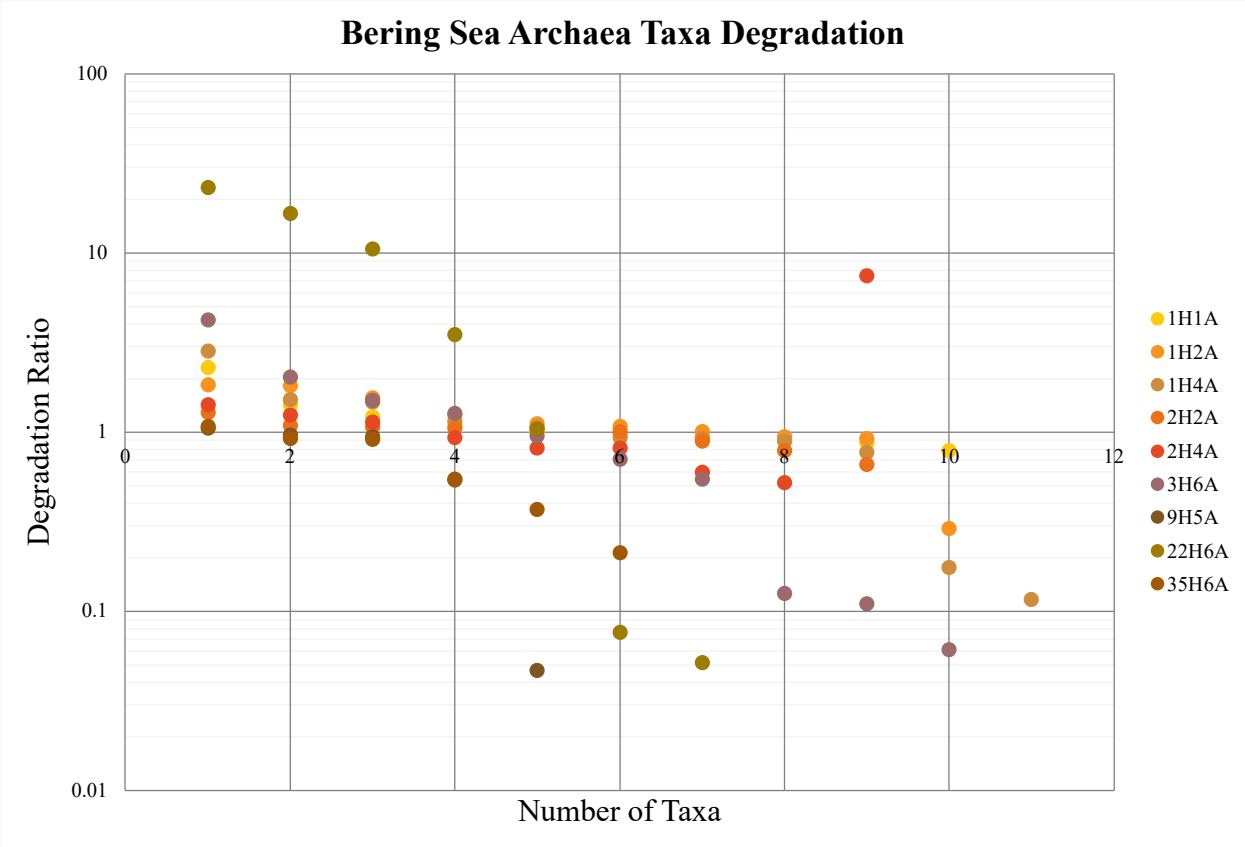

Figure 9. Archaeal S-curve represents the degradation ratio for paired sediment samples. Each dot represents one OTU.